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THE VELIGER

Development of *Tylodina fungina* Gabb, 1865 (Gastropoda: Notaspidea) from the Pacific Coast of Panama

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Abstract. The biology of notaspidean gastropods is not well studied and the development of tylodinoids is almost entirely unknown. Here I report observations on the reproduction and development of *Tylodina fungina* (Gabb, 1865) from the Perlas Islands on the Pacific coast of Panama. This species lives, feeds, and lays flat egg ribbons on the verongid sponge *Suberea azteca* (Goméz and Bakus, 1992). The egg ribbons contain hundreds of rows of 80 μm eggs, each singly encapsulated in a round 125 μm capsule. The ribbon also includes strings of extra-capsular material which is unevenly distributed through the mass. The eggs have equal cleavage and the ciliated "trochophore" stage is followed by an encapsulated veliger, which has a large, dark-red pigmented mantle organ. At hatching the transparent, left-handed larval shell is 123 μm long, and each semicircular velar lobe is unpigmented. There is a distinct operculum, but the eyes and tentacles have not developed. After 3 weeks in culture the larvae had reached a shell length of 162 μm and still had no eyes or tentacles. The larvae did not survive to settlement.

Key Words: Tylodinidae, Notaspidea, extra-capsular yolk

INTRODUCTION

The opisthobranch superfamily, Tylodinoidea, contains notaspideans with an external, limpet-like shell. The superfamily consists of 2 families, the Umbraculidae and the Tylodinidae. Umbraculidae is monotypic with a single species with a worldwide distribution, and the Tylodinidae is comprised of two genera; *Tylodina* with 5 species and *Anidolyta* with 2 or possibly 3 species (Willan, 1987). *Tylodina* is considered "primitive" in the notaspideans. However the monophyly of the notaspideans is contentious, with some families possibly including the sister-group of the nudibranchs (Wägele and Willan, 2000). Therefore data on any notaspideans could be useful in testing their monophyly as well as helping to reconstruct character state evolution within the opisthobranchs.

Although representatives of both families of tylodinoids have been examined morphologically, the biology of most of the species remains largely unstudied and the life history is not known for any species of *Tylodina* (Willan, 1998; Gibbson, 2003). One species each of the five described species of *Tylodina* occurs in Australia, South Africa, the Mediterranean, western Atlantic, and Tropical East Pacific. This unusual biogeographic pattern and the fact that the species are diagnosed with few subtle anatomical features has lead to suggestions that all species of *Tylodina* should be synonomized under the name *T. perversa* (Thompson, 1970). While disagreeing with this extreme view, Willan (1998) suggests that developmental data could be useful in further demonstrating the validity of the *Tylodina* species.

The only published developmental information for the superfamily is limited to the following observations of *T. corticalis* and *Umbraculum sinicum*, each reported for a single spawn of a single female by Thompson (1970) and with some additional information from one other individual of *U. sinicum* from Ostergaard (1950). *Tylodina corticalis* is reported to have a bright yellow spiral egg ribbon that is attached flat to the substrate and to contain eggs 98 μm in diameter. *Umbraculum sinicum* deposited a coiled ribbon that contained egg capsules 480–500 μm in diameter. Each capsule contained 30–45 eggs, which were 80–90 μm in diameter. Larvae hatched with statocysts and a distinct operculum but without eyes. A pigmented mantle organ is evident from Figure 32 in Ostergaard (1950). No other observations of the embryology or larval type have been published for the entire superfamily. Here I describe the embryology and larval development of *Tylodina fungina* as a step towards documenting development in this phylogenetically important group.

MATERIALS AND METHODS

12 adult *Tylodina fungina* were collected by dredging in the Perlas Isands (8°35.9′N, 78°1.0′W and 8°16.0N, 79°1.3W) during February and April 2007. The snails were brought to the surface attached to the host sponge

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Suberea azteca (Goméz and Bakus, 1992). *Tylodina fungina* is usually reported associated with *Aplysina fistularis*, which appears superficially similar to *S. azteca*. Identification of *S. azteca* was verified from a preparation of skeletal material and comparison with the original species description. The snails and some host sponge were kept in running seawater at ambient temperature (22–26°C). The sponge survived for 2 weeks under these conditions, but the snails survived for up to 6 weeks. Portions of egg ribbon were scraped from the surface of the containers and collected from the sponge skeletons and maintained in fingerbowls in the laboratory at 21–23°C. The water was changed daily and larvae were collected immediately upon hatching. After hatching the larvae were transferred to finger bowls with 1 µm filtered water. The water was changed every 2–3 days and larvae were fed *Isochrysis galbana*. The hydrophobic larvae were kept from getting stuck in the surface tension of the water by the addition of a few flakes of cetyl alcohol. Only uncleaved eggs and naturally hatched larvae were measured.

RESULTS

In the laboratory, the adult *Tylodina fungina* remained closely associated with the live sponge and were frequently observed feeding on it (Figure 1A). One of the sponges had been completely consumed by the snails and all that remained was the spongin skeleton. This skeleton was covered with egg ribbons (Figure 1B), giving the appearance of badly damaged sponge tissue when, actually, no sponge tissue remained on the skeleton. The three other sponges that remained largely intact showed eroded areas which each housed a snail (Figure 1A). Egg ribbons were not evident on these sponges, which suggests that egg production commences after depletion of the food supply. After the sponges died the snails deposited egg ribbons on the containers in which they were housed (Figure 1C).

The bright yellow egg ribbons were attached flat against the substrate and were arranged in an irregular spiral when laid on a smooth surface (Figure 1C). Those that were attached to the sponge skeleton were irregularly twined around the skeleton and incorporated portions of the skeletal fibers (Figure 2A). The 80.5 µm (n = 29, s. d. = 1.4 µm) eggs were yellowish cream-colored and were each contained within a 125.1 µm capsule (n = 19; s.d. 3.5 µm). These capsules are embedded in rows within the gel of the egg ribbon. Between the rows of egg capsules there were bright yellow streaks of extracapsular material (Figure 2 B–F). These streaks were inconsistent in width and were absent from some portions of the egg ribbon, but when present there tended to be 2 rows of eggs between each streak (Figure 2). At high magnification the streaks could be seen to consist of numerous tiny droplets (Figure 2F), which remained in the gel after hatching.

Table 1

Developmental time table of *Tylodina fungina* at 21–24°C.

Time	Stage
24 hrs	Blastula
48 hrs	Gastrula
4 days	"Trochophore"
6 days	Shell begins to develop
7 days	PMO develops
13–15 days	Hatching

Several egg ribbons were collected prior to first cleavage and were observed until hatching. A developmental schedule is given in Table 1. The two polar bodies remain associated with the eggs at least until gastrulation (Figure 3D). The first two cleavages appear to be equal and synchronous and there is no polar lobe (Figure 3B, C). By the beginning of the third cleavage division, one of the 4 cells is already slightly ahead of the others. Later, cleavage becomes more asynchronous and eventually forms a compact, animal-vegetally flattened blastula (Figure 3D). The gastrula is horseshoe shaped and appears to have been formed at least partially by invagination (Figure 3E). A trochophore-like stage with a distinct raised ring of cilia around the anterior end (Figure 3F) follows gastrulation. The pre-hatching veliger shows a distinct foot with an operculum (Figure 3G) and pair of statocysts and a large, pigmented mantle organ (PMO) on the right side (Figure 3G, H). The PMO appears black with epi-illumination and is dark red under transmitted light.

At hatching the larvae have a round, transparent shell 123.1 µm (n = 58 from 3 ribbons; s. d. = 6.1) in length with a single slightly left-handed whorl. On living larvae the shell appears smooth, but slight granular sculpture is evident on dead shells. The velum is un-pigmented and consists of two small, equal, semicircular lobes (Figure 3H). The operculum is present and the foot is simple. After 3 weeks the larvae had grown to 161.8 µm (n = 7; s. d. = 8.3) but still had not developed eyes or tentacles and showed no signs of competency to settle. The larvae survived for at most 4 weeks in culture. Despite repeated attempts to culture them, it was not clear why they failed to thrive.

DISCUSSION

As previously noted by Robertson (1985), developmental features have the potential to contribute useful data to understand high-level gastropod relationships. The main drawback to using developmental features is

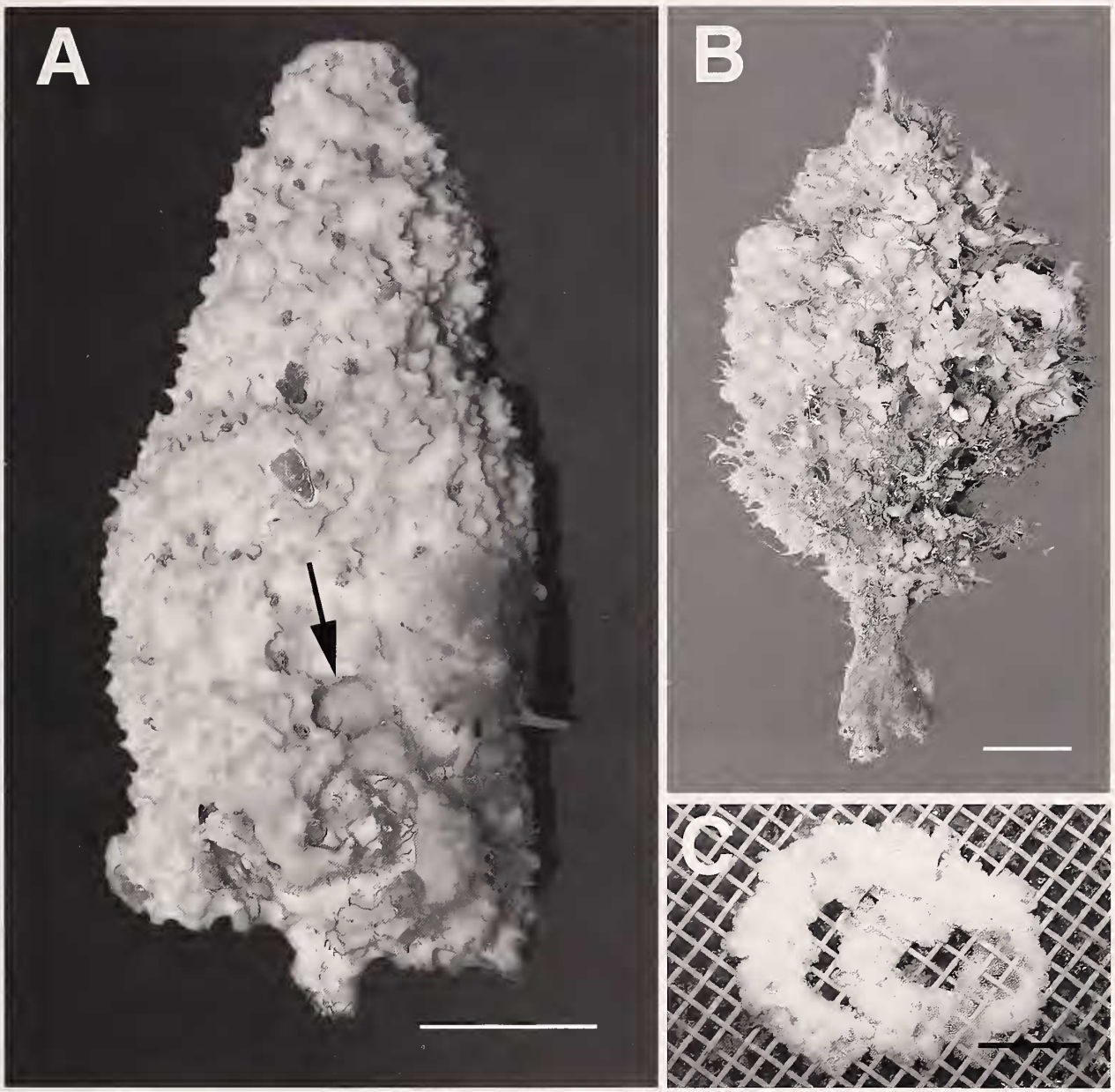

Figure 1. Adult *T. fungina* with host sponge and egg masses. **A.** Two adult *T. fungina* on their host sponge. The smaller individual (arrow) is sheltered in a depression in the ectosome of the sponge. Scale = 4 cm; **B.** Skeleton of the host sponge covered with egg masses of *T. fungina*. Scale = 2 cm; **C.** Egg mass of *T. fungina* deposited on a plastic mesh. This mass is smaller and more tightly coiled than most of the masses deposited on flat surfaces. Scale = 1 cm.

that few data are available for many interesting groups. Tylodinoids are a prime example of a phylogenetically important group where little is known. However, some comparisons of the egg masses can be made with previously published observations.

The egg masses of *T. fungina* as described here seem generally similar to those of the Australian congener, *T. corticalis*, with a flat ribbon attached in a coil to the substrate. Egg masses of both species are yellow, but contain cream-colored eggs (Thompson, 1970), suggesting that *T. corticalis*, like *T. fungina*, deposits extracapsular material in the egg ribbons. The difference in egg size between the 80 µm eggs of *T. fungina* and the 98 µm eggs of *T. corticalis* further bolsters their status as distinct species. The lack of information on *Umbraculum* species makes it difficult to determine how consistent the egg masses are throughout the family. The large number of eggs per capsule in *Umbraculum*

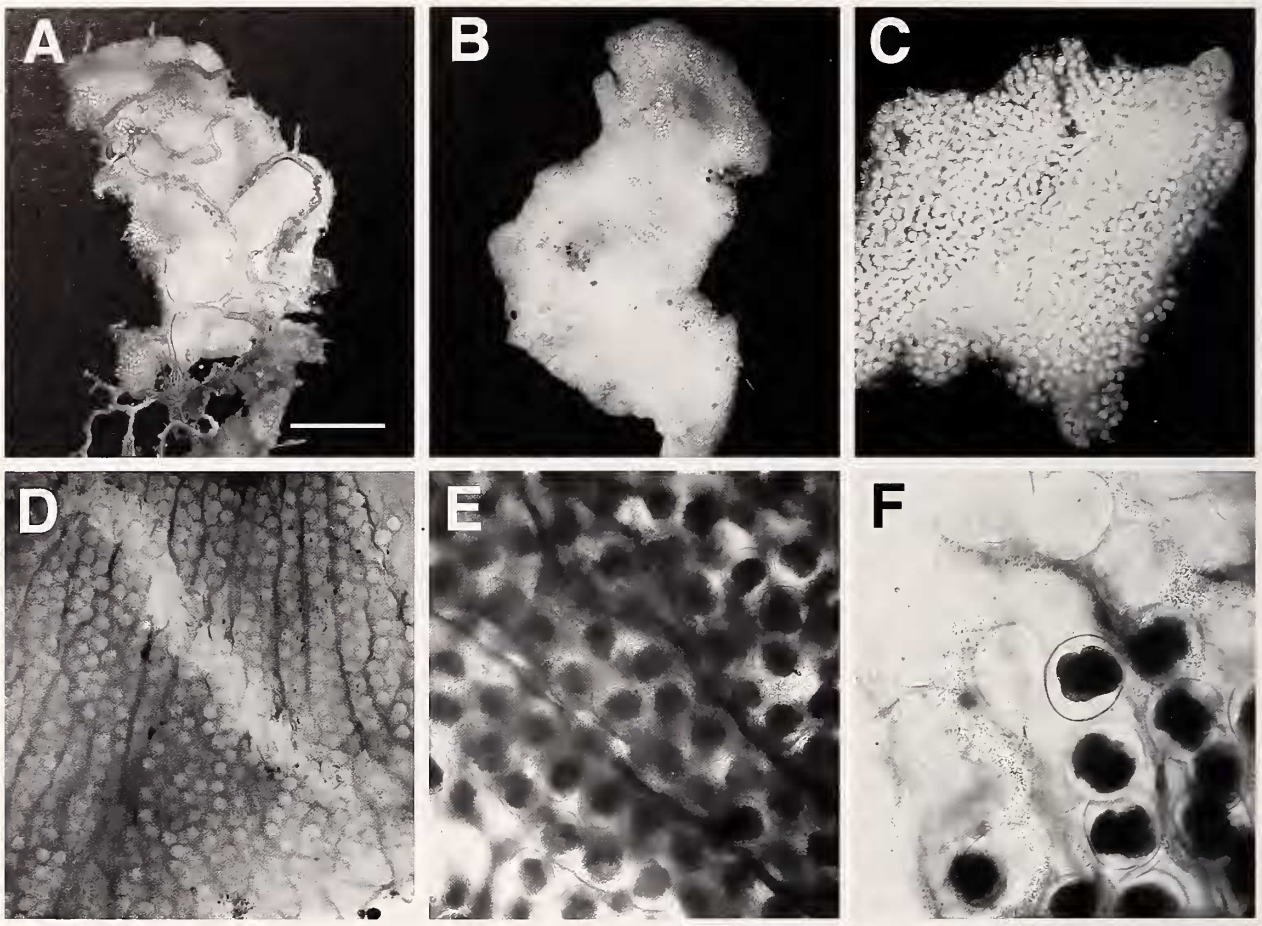

Figure 2. Egg ribbons of *T. fungina*. **A.** Egg ribbon attached to the sponge skeleton; Scale = 1 cm; **B.** Egg ribbon that was laid on a smooth surface, the lines of extra-capsular material are clearly visible in color but difficult to see in black and white. Scale = 1 cm; **C.** The multiple layers of eggs and the uneven distribution of the extracapsular material are can be seen. Scale = 5 mm; **D.** and **E.** Closer views showing the arrangement of eggs in two rows between each string of opaque extracapsular material. Scale = 1.5 mm and 500 μm respectively; **F.** Detailed view of egg capsules embedded in the gel and the droplets of extracapsular material. These droplets remain in the gel after hatching. Scale = 150 μm.

sinicum (Thompson, 1970) does show that there are some differences.

Unlike the Tylodinoids, there is considerable published information on the development of the other notaspidean superfamily, the Pleurobranchoidea (reviewed in Gibson, 2003). Gibson (2003) described the typical features of notaspidean development on the basis of her detailed observations of the development of *Pleurobranchaea maculata* and a review of the literature. These new observations of *Tylodina* development suggest that tylodinid development may differ significantly from pleurobranchid development. Unlike pleurobranchids, tylodinids have a larval operculum and extracapsular material (Table 2). Unfortunately, the larvae in this study did not survive long enough to determine if the larval mantle overgrows the larval shell (an unusual characteristic of pleurobranchids). It is unlikely, however, that this would happen as adult Tylodinids, unlike pleurobranchids, have a fairly large external shell that is not covered by the mantle. It may be that the mantle overgrowth of the larval shell is what prevents pleurobranchid larvae from being hydrophobic, like other opisthobranch veligers.

The most unusual characteristic of the *Tylodina fungina* egg masses was the presence of extracapsular material. Similar material in opisthobranch egg ribbons is usually referred to as "yolk" in the literature, although there is usually little evidence beyond a similar color that suggests this material is indeed yolk. "Yolk bodies" embedded in the egg ribbon jelly outside the egg capsules are well known for tropical chromodorids and sacoglossans (Boucher, 1983). Boucher (1983) described three kinds of extra-capsular material. Chromodorids have yolk that is present as either cap-

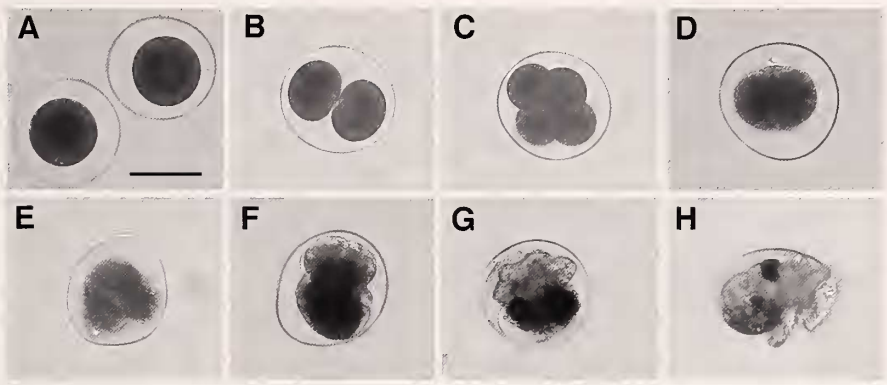

Figure 3. Developmental stages of *T. fungina*. **A.** Uncleaved eggs individually enclosed in transparent capsules, which have been removed from the gelatinous mass. **B.** 2-cell stage, the polar bodies are not visible. **C.** 4-cell stage. **D.** The flattened blastula with the polar bodies marking the animal pole. **E.** Horse-shoe shaped gastrula **F.** Late "trochophore"stage with the beginnings of the shell showing on the bottom right **G.** Encapsulated veliger, 2 days prior to hatching. The red PMO is visible on the animal's right and has not yet developed the black pigmentation. The velum, foot, and operculum are all well developed. **H.** 2-week old veliger larva. Scale = 100 μm.

like "yolk bodies" associated with individual capsules or discrete "yolk" masses distributed through the egg mass. *Elysia* species have strings of "yolk" running through the egg masses (Boucher, 1983). The overall morphology of *Elysia* egg masses is strikingly similar to those described here for *T. fungina* (P. Krug, pers. com). It has yet to be determined if the material included in the *T. fungina* egg masses is yolk, but it seems unlikely. The material is a different color (bright yellow) from the eggs (cream) and remains in the gel of the egg mass after hatching. The presence of this "yolk" in several other species with planktotrophic development, where the larvae are not retained near the egg mass after hatching (Boucher, 1983) suggests that this material might not have a nutritive function. There is some circumstantial evidence that the function in *Tylodina* might be defensive. Becerro et al. (2003) showed that egg masses and extracts of egg masses from *Tylodina perversa* deter feeding by damselfish with the same efficiency as the chemically defended adult snails and Ebel et al. (1999) showed that defensive chemicals are sequestered in the egg masses of the same species. Detailed examination of this material is necessary before their function can be determined.

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Table 2

Comparisons of Tylodinid and Pleurobranchid development.

Character	Tylodinids	Pleurobranchids
Egg masses	Flat ribbons	Strings
Extra-capsular material	Present	Absent
Extra-embryonic, intra-capsular yolk	Absent	Present sometimes
Type 1 larval shell	Present	Present
Larval Shell	Hydrophobic	Not hydrophobic in *Pleurobranchaea maculata* ‡
Larval shell growth	No observations of mantle-over growth *	Over-grown by mantle
Operculum	Present	Absent §
PMO	Present	Present
Larval eyes	Absent at hatching	Absent at hatching

‡ Glenys Gibson, pers. com. 2007

* More data is necessary to verify this observation.

§ Reported as absent in the group by Gibson (2003), but curiously Ostergaard (1950) reported opercula on 2 species of Pleurobranchids. Opercula were not present in other published studies of development in this group.

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